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Notes and Comments

Mitochondrial DNA in Ancient Amerindians

Maria Victoria Monsalve

Department of Biological Anthropology, University of Cambridge, Cambridge CB2 3DZ, United Kingdom
e-mail: mvm20@cus.cam.ac.uk

A recent study (Ribeiro-dos Santos et al., 1996) presents the results of the sequencing of the D-loop region of mtDNA in bone fragments from skeletons of pre-Columbian Amerindians. The authors claim in the September 1996 issue of the *American Journal of Physical Anthropology* that their study of mitochondrial DNA (mtDNA) sequencing from ancestral Amerindian populations of the South American continent is the first of its kind.

However, there was an earlier study, published in the July 1996 issue of the *Annals of Human Genetics*, which showed the results of sequencing of mtDNA from pre-Columbian mummies from Colombia, South America. This study also surveyed for the presence of the four Native American haplotypes, using PCR and restriction enzyme analysis (Monsalve et al., 1996). A summary of the sequence results from the control region of mtDNA from these mummies was published in 1994 (Monsalve et al., 1994a).

These studies used specimens that belong to the collection of the Instituto Colombiano de Anthropologia, Bogota (Colombia), and the entire collection of the Museu Paraense Emilio Goeldi, Para (Brazil), respectively. The carbon dating of the Colombian mummies indicated ages between 150 and 1,526 years BP. Three of the mummies were from Chiscas, Pisba and Socota, in the Boyaca region (Colombia), and belonged to the Lache, Tunebo and Muisca ethnic groups, respectively. The other three mummies were of unknown origin and their ethnic group is unknown. The skeletons studied by Ribeiro-dos Santos et al. (1996) were excavated from 11 different archaeological sites in the Brazil Amazon region, states of Para, Amapa and Amazonas with dates estimated at 500–4,000 years BP.

The extraction of soft tissue DNAs derived from diaphragm, abdominal muscle, intestine or muscle covering the ribs of six mummies, and the extraction of hard tissue DNAs derived from fragments of bones, was done with special care to avoid contamination with contemporary DNA. Both laboratories followed procedures to reduce the possibility of contamination, as recommended (Paabo et al., 1990). For example, they used gloves and masks during sample manipulation, and to prevent aerosol contamination between solutions the DNA samples were manipulated with pipettes equipped with filter-plugged pippette tips. DNA extractions of soft and hard tissues were carried out in duplicate for each sample. As negative controls, the extraction protocols were carried out on the buffer alone. Additionally for the soft tissues, DNA was extracted from *E. coli* as previously reported (Monsalve et al., 1994b).

Different approaches were used by the two groups for the amplification and sequencing of the mtDNA control region. Thus, for the soft tissues, the amplified DNA fragments were 169, 228 and 352 bp, using four primers and overlapping DNA sequences, covering the region between L16049 and H16401. For the hard tissues, the final amplified fragment of 424 bp was derived from an initial amplification of a 598 bp fragment. To purify PCR products away from non-specific amplification products, low melting agarose gel and 0.8% agarose gel were used, respectively. DNA was sequenced using the Taq DyeDeoxi Terminator Cycle Sequencing Kit (Applied Biosystems, Foster, CA) for the soft tissue DNA template, while the sequencing

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for the hard tissue DNA templates employed the Sequenase sequencing kit (U.S.B.) with α(35S)dATP labelling.

A comparison of nucleotide sequence substitutions revealed by the studies shows several number of identical transitions at positions 16111, 16290, 16327, 16298, 16325 and 16362 and 16319. The transitions at positions 16290 and 16311 have been found in Asians (Horai and Hoyasaka, 1990; Stoneking et al., 1991; Torroni et al., 1993b). The substitution 16327 has been found in Asians (Stoneking et al., 1991; Torroni et al., 1993b). The substitutions (T→C) at positions 16298 and 16325 were found in Asians (Stoneking et al., 1991). Finally the transitions at positions 16362 and 19319 have been found in Asians (Horai and Hayasaka, 1990; Stoneking et al., 1991; Shields et al., 1993).

Interestingly both studies found haplotypes previously not identified in Native Americans. Most of the haplotypes found in the mummies and in the skeletons seem to belong to one of the haplotypes that delineate Native Americans (Torroni et al., 1992). However, one of the haplotypes found in the mummies (MO), and haplotypes M08 to M13 found in the skeletons, do not have all the substitutions that characterize the four Native American founder haplotypes.

The existence of additional haplotypes in contemporary Amerindians (Baillet et al., 1994) and pre-Columbian Amerindians (Stone and Stoneking et al., 1993) has been reported. Recently, three new founder Native American haplotypes have been indicated (Easton et al., 1996). The finding of new haplotypes in one of the mummies and in the skeletons could indicate additional Amerindian haplotypes that have not been detected in contemporary Amerindians.

The possibility of erroneous results in studies of ancient DNA due to contamination with contemporary DNA is well known (Hagelberg and Clegg, 1993; Handt et al., 1994). A comparison of both studies shows a high similarity of substitutions in the mtDNA control region. The substitutions found in both studies and the restriction enzyme analysis in the mummies indicated mainly Amerindian haplotypes (Torroni et al., 1993a). It is unlikely that the results of both studies are due to contamination since they were done in different laboratories and contact or manipulation of the samples in Colombia and Brazil was done on different people.

In summary, the results found in the mtDNA sequences of the mummies are validated by the findings in skeletons, and vice versa. This is the most important consideration, regardless which research group was the first to study mtDNA sequences of ancestral Amerindian populations of South America. Colombia and Brazil are populated by different ethnic Amerindian groups, who share mtDNA features.

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